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## Do algae blooms dilute the risk of trematode infections in threespine sticklebacks?

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**Abstract** Human-induced growth of macro-algae is often assumed to increase trematode infections in fishes by increasing the abundance and condition of the parasite's intermediate host – snails – as this can boost the release of trematode larvae, cercariae, from the intermediate hosts. However, macro-algae can also impose barriers to the transmission of cercariae and reduce infections. We investigated whether an increased growth of filamentous algae affects the transmission of *Diplostomum pseudospathaceum* cercariae to the threespine stickleback *Gasterosteus aculeatus*, a common fish in eutrophied shallow waters. We exposed sticklebacks to trematode cercariae in the absence and presence of artificial filamentous algae, and recorded effects on the proportion of sticklebacks infected and the number of encysted metacercariae per fish. No significant effect of artificial algae on cercariae transmission was detected. However, the body size and the sex of the sticklebacks were strongly correlated with the number of encysted metacercariae per infected fish, with females and larger individuals being more infected. We discuss different factors that could have caused the difference in parasite transmission, including sex-related differences in body size and behaviour of sticklebacks [Current Zoology 61 (6): 991–995, 2015].

**Keywords** Anthropogenic disturbance, Cercariae, Dilution effect, *Diplostomum pseudospathaceum*, Eutrophication, Filamentous algae, Parasites, Physical interference

Human disturbance of the environment often triggers disease outbreaks by altering the dynamics of host populations (Lafferty and Kuris, 2005). However, changes in the density of non-hosts can also shape epidemics by diluting the infection risk (Johnson and Thieltges, 2010; Ostfeld and Keesing, 2012; Thieltges et al., 2008). A dilution effect can arise from any mechanism impairing parasite transmission, such as the presence of non-competent (decoy) hosts, predation or hyperparasitism. For example, the physical presence of non-host organisms can act as a physical barrier and prevent short-lived free-living stages of parasites from reaching their host. For instance, Prinz et al. (2009) found the presence of seaweeds to physically obstruct the transmission of free-living trematode stages to their host, the blue mussel *Mytilus edulis*. This prevented the successful encystment of the parasite in and on mussels. Such results suggest that human-induced eutrophication of aquatic ecosystems that promotes the growth of aquatic vegetation could impose a dilution effect and hamper parasite transmission (Smith et al., 2006; Thieltges et al., 2008). Vegetation could influence parasite transmission both directly, by forming physical barriers to the movement of free-living parasites, such as the transmission stages

of trematodes and other endohelminths (Christensen, 1979; Prinz et al., 2009), and indirectly by altering the behaviour of the parasite or the host, such as their activity and movements. Yet, surprisingly little attention has been given to the influence that human-induced growth of aquatic vegetation has on parasite transmission rate.

In the Baltic Sea, human-induced eutrophication is currently promoting the growth of filamentous algae, such as the green algae *Chladophora glomerata* (Krause-Jensen et al., 2009). The algae could act as barriers to the transmission of free-living stages of parasites to their hosts. For instance, the transmission of free-living stages of the eye fluke *Diplostomum* sp. to fishes could be hampered by an increased algal density. The trematode reproduces asexually in aquatic snails – their primary intermediate host – from which hundreds of free-living cercarial stages can be released per day. These seek to infect fishes – their secondary intermediate hosts. The algae could also influence the behaviour of their fish hosts, for instance by influencing the time they spend hiding in the vegetation and their activity pattern. A common fish host in the Baltic Sea is the threespine stickleback *Gasterosteus aculeatus*. Previous studies have suggested that eutrophication promotes trematode

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infections in fishes by facilitating the growth of snail populations and, in so doing, the release of cercariae from the snails (Johnson and Chase, 2004; Johnson et al., 2007). Accordingly, Heuschele and Candolin (2010) found threespine stickleback males nesting in dense vegetation to be more likely to carry metacercariae of *Diplostomum* sp. than males nesting in sparse vegetation. However, the physical presence of filamentous algae could also interfere with the transmission of cercariae and lessen the promoting effect of algae on infection rate. In the present study, we investigate if an increased growth of filamentous algae interferes with the transmission of *Diplostomum* sp. cercariae to threespine sticklebacks. Such interference could mitigate increases in trematode infections in eutrophied habitats.

## 1 Materials and Methods

### 1.1 Collecting, rearing and husbandry

Threespine sticklebacks were raised in the lab, from adult threespine sticklebacks caught during the breeding season from the littoral of the Baltic Sea in South-western Finland (60°N, 23°E). The salinity in the area is low, 5.5 ppt, and sticklebacks are often infected with diplostomid trematodes (Heuschele and Candolin, 2010). Fertilized eggs were divided into two subfamilies and incubated at 18°C in aerated 10-L tanks. The juveniles were first fed newly hatched artemia nauplii and later small chironomid larvae. When the juveniles were 30 days old, the subfamilies were transferred to 60 × 40 × 30 cm tanks and maintained at 12: 12 h light: dark cycle at 16°C, and fed twice a day with defrosted chironomid larvae. Densities across subfamilies were adjusted to be approximately equal, gradually decreasing as the fish grew bigger. The fish were kept in the tanks until used in the experiment.

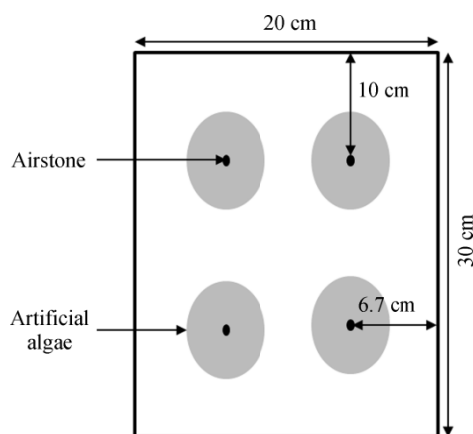
Great pond snails *Lymnaea stagnalis*, naturally infected with *D. pseudospathaceum*, were collected from a lake in Germany (see Kalbe and Kurtz, 2006) in early September 2012, when cercarial shedding is at its highest. The snails were transported to the Viikki campus at University of Helsinki where they were kept under the same temperature and light regime as the sticklebacks. They were fed *ad libitum* with fresh lettuce.

### 1.2 Experimental infections

To obtain *D. pseudospathaceum* cercariae for the experiment, five snails were transferred to individual glass jars filled with 50 ml of dechlorinated tap water and exposed to bright light for 90 min (Kalbe and Kurtz, 2006). The five cercarial suspensions were combined and 100 cercariae of *D. pseudospathaceum* were trans-

ferred into a Petri dish. After 90 minutes, the Petri dish was immersed into a 4-l tank filled with dechlorinated tap water (Kalbe and Kurtz, 2006) and submitted to one of two treatments: presence or absence of artificial filamentous algae. To standardize conditions and investigate the influence of the physical presence of the algae, without confounding effects of photosynthesis, exudates or the presence of other organisms, artificial filamentous algae was used. This consisted of two grams of dry aquarium filtering wool attached to four air stones. The wool was stretched to cover about 15% of the tank volume (Fig. 1), which mimics the growth of filamentous algae in eutrophied coastal waters of Southern Finland (personal observations).

Before the start of the experiment, the four air pumps were activated for 2 min (airflow: 1 l.min<sup>-1</sup>) to distribute the cercariae within the tank. Five minutes later, a stickleback was introduced at a random position in the tank. Two days later (48 h), the stickleback was removed. The time period ensured that all cercariae penetrating the skin had time to encyst as metacercariae in the eyes: the optimum age of infectivity for *D. pseudospathaceum* is within the first few hours of release from snails (Whyte et al., 1991), with encystation no longer occurring when cercariae are 12 hours old (Karvonen et al., 2003), and migration to the eye and encystation of the parasite taking up to 24 hours after penetration of host tissues (Ratanarat-Brockelman, 1974). The standard length (SL) of the stickleback was measured, after which it was sacrificed by decapitation and its sex determined. Its eyes were screened for metacercariae of *D. pseudospathaceum*, which were extracted from the eye lens and counted. Fifty-four sticklebacks were tested. The assignment of the sticklebacks to the two treatments was homogenised according to sex and family.



**Fig. 1** The experimental aquarium

Artificial filamentous algae were present only in the algal treatment.

### 1.3 Data analysis

Only replicates where the fish remained alive until the end of the experiment were included in the analysis (one fish died because of unknown reason during the 48 h trial). Data analysis was performed using generalized linear mixed models, using lme4 and nlme packages of the software R (version 2.15.2, R Development Core Team 2012). Family was included as a random factor and size (SL), sex and treatment (artificial algae) as fixed factors. When investigating the effect of artificial algae on the probability of infection (infected vs. non-infected), models were fitted using a binomial error distribution. When estimating the impact of artificial algae on the mean abundance of metacercariae per stickleback, the number of parasites was square-root transformed and models were fitted using normal error distribution. Backward model selection was conducted using Akaike information criterion (AIC). Due to collinearity between sex and size, one of these two variables at a time was included in the initial model. The distribution of the residuals was checked visually following Zuur et al. (2009).

## 2 Results

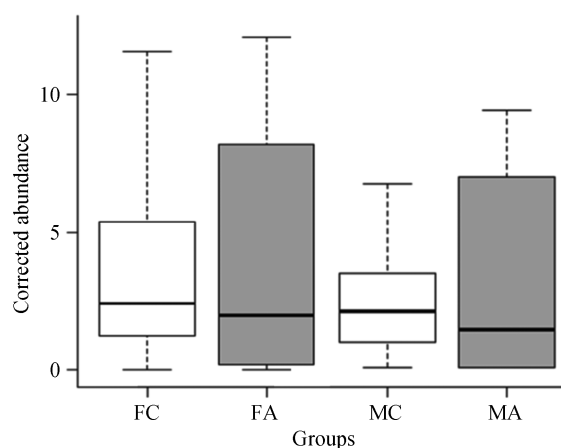
In total, nine out of 53 fish did not carry any metacercariae of *D. pseudospathaceum* (two out of 26 in the control treatment and seven out of 27 in the algae treatment). No statistically significant effect of artificial algae, size (SL) or sex on the probability of infection was found (SL:  $n = 53$ ,  $z = 0.70$ ,  $P = 0.49$ ; Sex:  $n_{\text{females}} = 29$ ,  $n_{\text{males}} = 24$ ,  $z = -1.55$ ,  $P = 0.12$ ; Treatment:  $n_{\text{control}} = 26$ ,  $n_{\text{artificial algae}} = 27$ ,  $z = -1.44$ ,  $P = 0.15$ ).

The presence of artificial algae had no significant effect on the number of encysted metacercariae (Table 1, Fig. 2). However, the number encysted increased with body size (Table 1) and was higher in females ( $n_{\text{females}} = 29$ ,  $n_{\text{males}} = 24$ ,  $t = -2.11$ ,  $P = 0.04$ ). Females were larger than males [SL males (mean  $\pm$  SD):  $44.0 \pm 3.4$  mm; SL females:  $49.4 \pm 2.9$  mm], and models including either standard length or sex scored very similar Akaike information criterion (AIC) values (AIC<sub>Algae + SL</sub>: 234.49; AIC<sub>Algae + Sex</sub>: 235.97). A power test (longpower package, Donohue et al., 2013) revealed that in order to detect an effect of artificial algae on number of encysted metacercariae, with a difference of 70% between algae treatments (effect size), which is comparable to the findings of Christensen (1979), a minimum sample size of 28 individuals per treatment is required to reach a statistical power of 0.80. As we had 26 and 27 individuals per treatment, the results strongly suggests that the

artificial algae had no significant effect on infections, but more research is needed to verify this.

## 3 Discussion

The presence of artificial algae had no significant effect on the probability of sticklebacks becoming infected with *D. pseudospathaceum*. Moreover, the size and sex of the sticklebacks did not correlate with infection probability. This is unsurprising, as the infection dose was high in order to ensure that most sticklebacks were infected (Kalbe and Kurtz, 2006). The number of parasites transmitted to sticklebacks, on the other hand, was correlated with the size and sex of the sticklebacks, but no significant effect of artificial algae was detected. The number of replicates is not high enough to refute the probability of an effect of artificial algae, but indicates that if there is an effect, the effect must be small and of less biological importance.



**Fig. 2** The influence of artificial algae on the number of *Diplostomum pseudospathaceum* metacercariae encysted in the eye of sticklebacks, corrected for host size by dividing the number of metacercariae by the size of the stickleback. FC = females in control treatment ( $n = 15$ ), FA = females in artificial algae treatment ( $n = 14$ ), MC = males in control treatment ( $n = 11$ ), MA = males in artificial algae treatment ( $n = 13$ ). Box plots show 10<sup>th</sup>, 25<sup>th</sup>, 50<sup>th</sup> (median), 75<sup>th</sup>, and 90<sup>th</sup> percentiles with horizontal lines.

**Table 1** The influence of artificial algae and the body size of the stickleback on the mean number of *Diplostomum pseudospathaceum* encysted in the eye

Fixed factor	Estimate $\pm$ SE	<i>t</i>	<i>df</i>	<i>P</i>
Intercept	-4.55 $\pm$ 3.29	-1.38	41	0.1740
Algae	-0.22 $\pm$ 0.52	-0.42	1	0.6754
Standard length	1.65 $\pm$ 0.69	2.39	41	0.0217

GLMM with Poisson error structure was fitted to the data, including 29 females (15 in control, 14 in algae treatment) and 24 males (11 in control, 13 in artificial algae treatment). Only the results for the fixed factors are presented.

The absence of a clear effect of artificial algae on infections suggests that anthropogenic eutrophication may not strongly limit the transmission of trematodes to sticklebacks through physical obstruction or alteration of the behaviour of the host or the parasite. We cannot separate between the effect of physical interference of transmission and changes in the behaviour of the host or the parasite, and the possibility remains that algae do hinder transmission but that a change in the behaviour of the fish or the parasite compensate for the change, resulting in no net effect. Moreover, the different origin of the parasite and the host populations could have influenced the results. Parasite populations co-existing with sticklebacks in the Baltic Sea could react differently to the presence of algae, particularly if the parasites in the Baltic Sea have not yet adapted to the recent increase in the growth of filamentous algae and, hence, are more sensitive to dense algae growth than the parasite population from the lake.

The effect of aquatic vegetation on the transmission of trematode cercariae appears to vary among systems, as some experimental studies find aquatic plants and algae to obstruct transmission (e.g. Christensen, 1979; Prinz et al., 2009), while one study does not (Hopper et al., 2008). It is possible that variation in the shape and structure of plants and algae influence their potential to physically interfere with parasite transmission. In the present study, the effect of artificial algae could differ from that of natural algae, as the texture of the filtering wool differs from that of natural filamentous algae. In the field, algae could also provide a substrate for other organisms that depredate on the cercariae, or the algae could alter water chemistry through photosynthesis and the release of chemical exudates, which in turn could influence cercariae transmission (Haas et al., 2002). Moreover, free-living cercariae of different trematode species vary in shape and swimming behaviour (see Santos et al., 2007), which could influence their sensitivity to algae barriers. Similarly, host species could differ in their behavioural responses to algal growth and, hence, in their susceptibility to infection in vegetation.

Our analysis revealed a correlation between parasite transmission and fish size and sex. The correlation with size could be a result of larger fish offering more surface for parasite penetration (see Poulin, 2000 and references therein). Females, on the other hand, could have carried more parasites than males because they were larger. However, as sex and size explained a similar proportion of the variation in infections, it is not possible to tease apart their possible influence in this study.

Behavioural differences between the sexes could have influenced infection rate, as earlier work has found sex-specific behavioural differences to influence parasite infections (e.g. Reimchen, 2001). More studies are needed to determine if sex-specific behaviour contribute to differences between the sexes in trematode infections also in the present system.

The influence of human-induced eutrophication on parasite infections is often complex, as environmental change can influence the parasite and the host directly as well as indirectly through effects on the community of species (Johnson and Carpenter, 2008). To unravel the influence of eutrophication on parasite infections, we need to consider the multitude of pathways in which eutrophication influences host-parasite interactions, including the influence of non-host organisms.

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